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Published in:
Journal of Neuroendocrinology

DOI:
[10.1046/j.1365-2826.2003.00986.x](https://doi.org/10.1046/j.1365-2826.2003.00986.x)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2003

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Citation for published version (APA):

Veenema, A. H., Meijer, O. C., de Kloet, E. R., & Koolhaas, J. M. (2003). Genetic selection for coping style predicts stressor susceptibility. *Journal of Neuroendocrinology*, 15(3), 256-267.
<https://doi.org/10.1046/j.1365-2826.2003.00986.x>

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Genetic Selection For Coping Style Predicts Stressor Susceptibility

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Key words: coping style, corticosterone, hippocampus, HPA axis, stress.

Abstract

Genetically selected aggressive (SAL) and nonaggressive (LAL) male wild house-mice which show distinctly different coping styles, also display a differential regulation of the hypothalamic-pituitary-adrenal axis after exposure to an acute stressor. To test the hypothesis that coping style predicts stressor susceptibility, the present study examined line differences in response to a chronic stressor. Chronic psychosocial stress was evoked using two paradigms. In the first paradigm, a SAL or LAL male was living in sensory contact (except tactile contact) with a dominant SAL male for 25 days (sensory contact stress). In the second paradigm, a SAL or LAL male was, in addition to the first paradigm, defeated by a SAL male for 21 consecutive days (defeat stress). The sensory contact stressor induced in LAL mice chronic body weight loss and increased plasma adrenocorticotrophic hormone levels compared to SAL mice and increased corticosterone levels, thymus involution and lower hippocampal mineralocorticoid receptor (MR): glucocorticoid receptor (GR) ratio compared to LAL controls. The defeat stressor increased corticosterone secretion and caused adrenal hypertrophy and thymus involution in both mouse lines. Defeated LAL mice showed long-lasting body weight loss and higher corticosterone concentrations than SAL mice and lower hippocampal MR: GR ratio and decreased immobility behaviour in the forced swimming test than LAL controls. Hypothalamic corticotropin-releasing hormone mRNA expression was higher in defeated SAL than in controls. The present data show that both stress paradigms induced line-dependent physiological and neuroendocrine changes, but that the sensory contact stressor produced chronic stress symptoms in LAL mice only. This latter stress paradigm therefore seems promising to analyse the role of genetic factors in the individual differences in stress-related psychopathology.

Wild house-mice selected for high and low aggression show profound differences in coping with environmental challenges. SAL (Short Attack Latency, high aggressive) mice display the 'active coping' style whereas LAL (Long Attack Latency, low to non aggressive) mice show the 'passive coping' style (1–4). This difference in coping style was recently found to be associated with a differential regulation of the hypothalamic-pituitary-adrenal (HPA) system under basal and acute stress conditions (5). The corticosterone output in LAL mice was found to be more sensitive to adrenocorticotrophic hormone (ACTH), but showed less day-night variation than in SAL mice. In addition, LAL mice showed a higher and prolonged stress-induced increase in plasma corticosterone compared to SAL (5). Furthermore, LAL mice have lower serotonin-1A (5-HT_{1A}) receptor expression and function in the hippocampus than SAL mice (6, 7).

Elevated circulating glucocorticoid concentrations and reduced hippocampal serotonergic function are considered hallmarks in

depression (8–10). This raises the question of whether LAL mice may be considered as a mouse model for susceptibility to depression. In this study, we tested the hypothesis that differences in coping style, as expressed in SAL and LAL mice, are a predictor for stressor susceptibility and the appearance of depression-like symptoms.

Two modified forms of the sensory contact model (11, 12) were used to evoke chronic psychosocial stress. In the first paradigm, a male mouse (SAL or LAL) was living in sensory contact (visual, auditory, and olfactory but not tactile contact) with a SAL male for 25 days (sensory contact stress). In the second paradigm, a SAL or LAL male was living opposite a SAL male for 25 days and was defeated by this dominant male for 21 consecutive days (defeat stress). Control male mice were housed in pairs with a female in a standard cage. The effects of the two stress paradigms were determined for behavioural and physiological parameters, HPA axis regulation [plasma corticosterone and ACTH, mRNA expression

of mineralocorticoid receptor (MR) and glucocorticoid receptor (GR) in the hippocampus and hypothalamic corticotropin-releasing hormone (CRH)] and binding to the 5-HT_{1A} receptor in the hippocampus.

Materials and methods

Mice

Two mouse lines, genetically selected for attack latency, originated from a colony of wild house-mice (*Mus musculus domesticus*) maintained at the University of Groningen, The Netherlands, since 1971. The mice were housed in perspex cages (17 × 11 × 13 cm) in a room with an artificial 12:12 h light/dark cycle (lights on from 00.30 h to 12.30 h). Standard laboratory chow and water was available *ad libitum*. The mice were weaned at 3–4 weeks of age, and were paired male-female at the age of 6–8 weeks. At the age of 92–100 days, male mice were tested for their attack latency as described previously (13). Briefly, SAL and LAL mice are confronted with a standard nonaggressive opponent in a neutral cage. The time it takes before a mouse attacks the nonaggressive opponent is measured on 3 consecutive days. The attack latency score is the mean of these daily scores. Neither SAL nor LAL mice experienced a social defeat. Only SAL mice with an attack latency of less than 50 s and only nonattacking LAL mice were used for the experiments. The SAL males came from the sixty to sixty-second generation of selection, the LAL males from the thirty-fifth to thirty-seventh generation and were aged 16 weeks (± 1 week). All experiments were in accordance with the regulations of the Committee for Use of Experimental Animals of the University of Groningen (DEC no. 2326).

Experimental procedure (Fig. 1)

Two modified versions of the sensory contact model (11, 12) were used to evoke psychosocial stress. In both paradigms (sensory contact stress and defeat stress), a LAL or SAL male was continuously living opposite another SAL male in a partition cage (75 × 29 × 27 cm) during the experimental period of 25 days. The perforated (diameter of 5 mm) transparent partition separated the cage into two equal halves and allowed the mice to see, hear and smell each other. In the sensory contact stress paradigm (SAL, *n* = 6; LAL, *n* = 6), physical contact was avoided during the whole experimental period. In the defeat stress paradigm (SAL, *n* = 5; LAL, *n* = 6), the partition was removed after 2 days of sensory contact, to allow physical interaction between the mice. After establishment of a dominant (SAL)/subordinate (LAL or SAL) relationship (in which the defeated mouse was subjected to 10 physical confrontations), the interaction was terminated by replacing the partition. This procedure took place once per day at unpredictable time points for 21 consecutive days. In both paradigms, after every third day, the experimental SAL and LAL males were moved to a novel partition cage and lived opposite, and/or were defeated by a different SAL male. The dominant SAL males remained in their own compartments. Control SAL (*n* = 8) and LAL mice (*n* = 7) were housed under standard conditions with a female in perspex cages of 17 × 11 × 13 cm and were moved to a novel cage every third day. Body weight was measured at days 0, 2, 5, 12 and 25 of sensory contact. At experimental day 25 (48 h after the last defeat), mice were decapitated under CO₂ anaesthesia between 08.00 h and 09.00 h, trunk blood was collected for corticosterone and ACTH measurements, brains were rapidly removed, quickly frozen in ice cold *p*-heptane and stored at –80 °C for subsequent

in situ hybridization and autoradiography, and several organs were removed and weighed.

Behavioural testing

Behaviour was measured 1 week before mice were placed in the partition cage (trial 1) and after 5 (trial 2), 12 (trial 3) and 23 (trial 4) days in the partition cage (i.e. after 3, 10 and 21 defeats, respectively). The elevated plus-maze and the sudden silence test were performed at least 2 h after the last defeat procedure. The following day, the open field and forced swim test were performed at least 2 h before the next defeat procedure. Behaviour in the elevated plus-maze, sudden silence test and forced swim test was recorded using the Observer, version 3.0 (Noldus Information Technology, Wageningen, The Netherlands).

Elevated plus-maze

The plus-maze was constructed from grey perspex and elevated to a height of 75 cm. It consisted of two open arms (30 × 5 cm) and two enclosed arms (30 × 5 × 15 cm, with a closed roof). The two open arms were surrounded by 4 mm-high ledges. The test took place in the early dark phase between 13.00 h and 15.00 h, under dim red light to encourage the mice to explore the maze. Each mouse was placed in the central square (5 × 5 cm) facing an open arm, and allowed to explore the maze for 5 min. The maze was cleaned thoroughly before each test. The percentage of time spent on the open arms [time on open arms/(time on open arms + time on closed arms) × 100], the percentage open arm entries (open arm entries/total entries × 100), and total number of entries were determined. An entry was defined as three of the four paws being on the arm.

Sudden silence test

Mice were placed in a large perspex cage (60 × 30 × 40 cm), within a soundproof wooden box with dim white light (5 lux) and a glass front enabling observation. The mice were exposed to a constant 70 dB background noise. This noise was switched off after 5 min and the duration of various behavioural elements was recorded during an additional 5-min period. In this test, it was expected that mice will react to the sudden silence with a brief period of freezing and orientation movements before they resume locomotion. The test was described and validated by Koolhaas *et al.* (14). The test took place in the early dark phase between 15.00 h and 17.00 h.

Open field

The open field test is widely used to measure anxiety-related behaviour in addition to general locomotor and explorative activity. The open field consisted of a round arena with a diameter of 90 cm, and surrounded by a wall 70 cm in height. At the start of the test, the mouse was transported to the arena in a clean and empty cage. The cage was covered by a perspex lid. The cage was then turned and put in the centre of the arena. The lid at the bottom was carefully removed and the cage was lifted, allowing the mouse to explore the arena for 5 min. Locomotion in the arena was recorded with a camera and automatically analysed with a software program Ethovision (Noldus Information Technology). The following parameters were determined: total distance travelled, immobility duration, velocity of movement, and thigmotaxis or wall-seeking behaviour. The test was performed in the late light phase between 09.00 h and 11.00 h, under white light conditions. The open field was cleaned thoroughly before each test.

Forced swim test

The present procedure was a modified version of the test described by Porsolt *et al.* (15). Briefly, mice were given a single trial in which they were forced to swim inside a narrow plexiglass cylinder (diameter of 10 cm) in soiled water for 5 min. The temperature of the water was 25 °C. The duration of immobility behaviour (floating in the water without struggling, making only those movements necessary to keep the head above the water) was recorded. The test was performed in the late light phase between 11.00 h and 12.00 h.

Radioimmunoassay for corticosterone and ACTH

Trunk blood was collected in chilled tubes containing EDTA for determination of corticosterone and ACTH levels. Blood samples were centrifuged at 2600 *g* for 10 min at 4 °C. Plasma samples were stored at –20 °C until assayed. Plasma corticosterone was determined in duplo using a commercially available radioimmunoassay (Mouse Corticosterone Radioimmunoassay Kit, ICN Biomedicals, Costa Mesa, CA, USA). The detection limit of the assay was 3 ng corticosterone/ml with an intra-assay variance of 4.4% and interassay variance 6.5%. A double-antibody radioimmunoassay (ACTH Radioimmunoassay kit, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) with intra-assay and interassay variances of

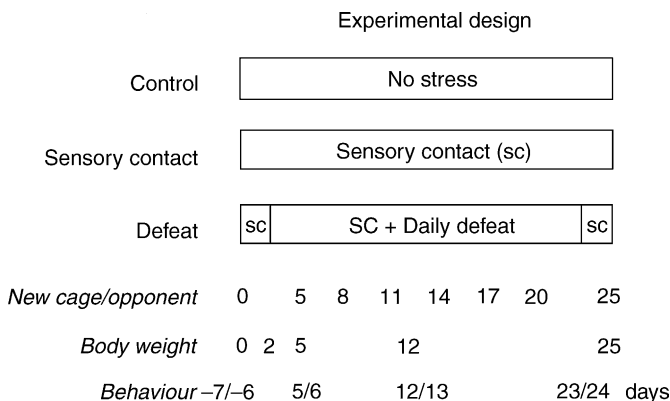


FIG. 1. Experimental design,

3.2% and 7.8%, respectively, was used to measure plasma ACTH. The detection limit of the assay was 1 pg ACTH/ml.

In situ hybridization

Brain tissue sections of 20 µm were cut on a cryostat and thaw-mounted on poly-L-lysine coated slides. These slides were stored at -80 °C until the time of hybridization. The hybridization protocol was adopted from Meijer *et al.* (16), with some minor modifications. Briefly, before hybridization, the sections were fixed in 4% paraformaldehyde, permeabilized by proteinase K treatment, acetylated twice with 0.25% acetic anhydride in 0.1 M triethanolamine and dehydrated in a graded ethanol series. Riboprobes were generated from linearized constructs containing the respective cDNAs in pBluescript. A 500-bp *Sall*-*HindIII* fragment of exon 2 of the mouse gene was used for GR and a 1.2-kb *NcoI*-*EcoRI* fragment of the mouse MR exon 2 for the MR (courtesy of Dr T. Cole). The cRNA from CRH was transcribed from a 1-kb cDNA insert in pGEM 4 containing full-length coding region of rat CRH. ³⁵S UTP labelled antisense probes were generated using the appropriate polymerase using a standard protocol. A hybridization mix was prepared containing 60% deionized formamide, 10% Dextran SO₄, 2 × SSC, 0.1 mg/ml yeast tRNA, 0.1 mg/ml sssDNA, 10 mM DTT, 0.05 M PBS. All radiolabelled probes were diluted to 20 × 10⁶ d.p.m./ml. 100 µl of these mixtures was applied to each slide, which was then covered with a coverslip. The sections were hybridized overnight in a moisturized chamber at 55 °C. The next day, coverslips were removed carefully and sections were washed in 2 × SSC for 10 min at room temperature. After washing, sections were treated with RNase A (2 mg/100 ml in 0.5 M NaCl, 0.1 M Tris, pH 7.5) at 37 °C for 10 min and subsequently washed at 55 °C in 2 × SSC for 10 min, 1 × SSC for 10 min, 0.1 × SSC for 2 × 30 min and, finally, at room temperature in 0.1 × SSC for 5 min. Sections were dehydrated in an ethanol series (70%, 80%, 96% and 100% ethanol) and dried on air. Hybridized slices were exposed to a X-Omat AR film (Kodak, Rochester, New York, NY, USA) for 3 weeks. Optical density was

determined by using an automatic image analysis system (Quantimet 500, Leica, Cambridge, UK). For GR and MR mRNA expression, the optical density of CA1, dentate gyrus (GR and MR), CA2 and CA3 (MR) were determined in three hippocampal sections of each mouse. The optical density of a small area between the CA1 and dentate gyrus was used for tissue background. The MR : GR ratio was determined per mouse by dividing the average value of the MR mRNA expression by the average value of the GR mRNA expression in the dentate gyrus and the CA1 region. To determine CRH mRNA expression, the optical density of one or two sections of each mouse containing the paraventricular nucleus (PVN) of the hypothalamus were measured. A nonhybridized region outside the PVN was measured for tissue background.

Autoradiography

Brain tissue sections of 20 µm thickness were cut on a cryostat and thaw-mounted on gelatine coated slides. These slides were stored at -80 °C until the time of radioligand binding. [³H]8-OH-DPAT binding to brain sections was performed according to Sijbesma *et al.* (17), with some minor modifications. Briefly, after 30 min preincubation at room temperature, the mounted sections were incubated in 0.17 M Tris-HCl, pH 7.6, containing 4 mM CaCl₂, 0.01% ascorbic acid and 10 µM pargyline in the presence of 1.5 nM [³H]8-OH-DPAT 2(*N,N*-di[2,3(*n*)-³H]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene, specific activity 221 Ci/mmol (Amersham TRK 850, Amersham, Bucks, UK) for 60 min at room temperature. Following incubation, the slides were washed in incubation buffer (2 × 15 min) at 4 °C and dried in a stream of cold air. Non-specific binding was determined in the presence of 1 µM 5-HT. Sections were exposed to 3H-sensitive film (Hyperfilm, Amersham TRK 850, Amersham, Bucks, UK) for 60 min at room temperature. For quantification, an automatic image analysis system (Quantimet 500, Leica) was used. The optical density in several brain regions was measured in two (dorsal raphe nucleus) or three sections (frontal cortex, hippocampus) and [³H]8-OH-DPAT binding was calculated in fmol/mg tissue.

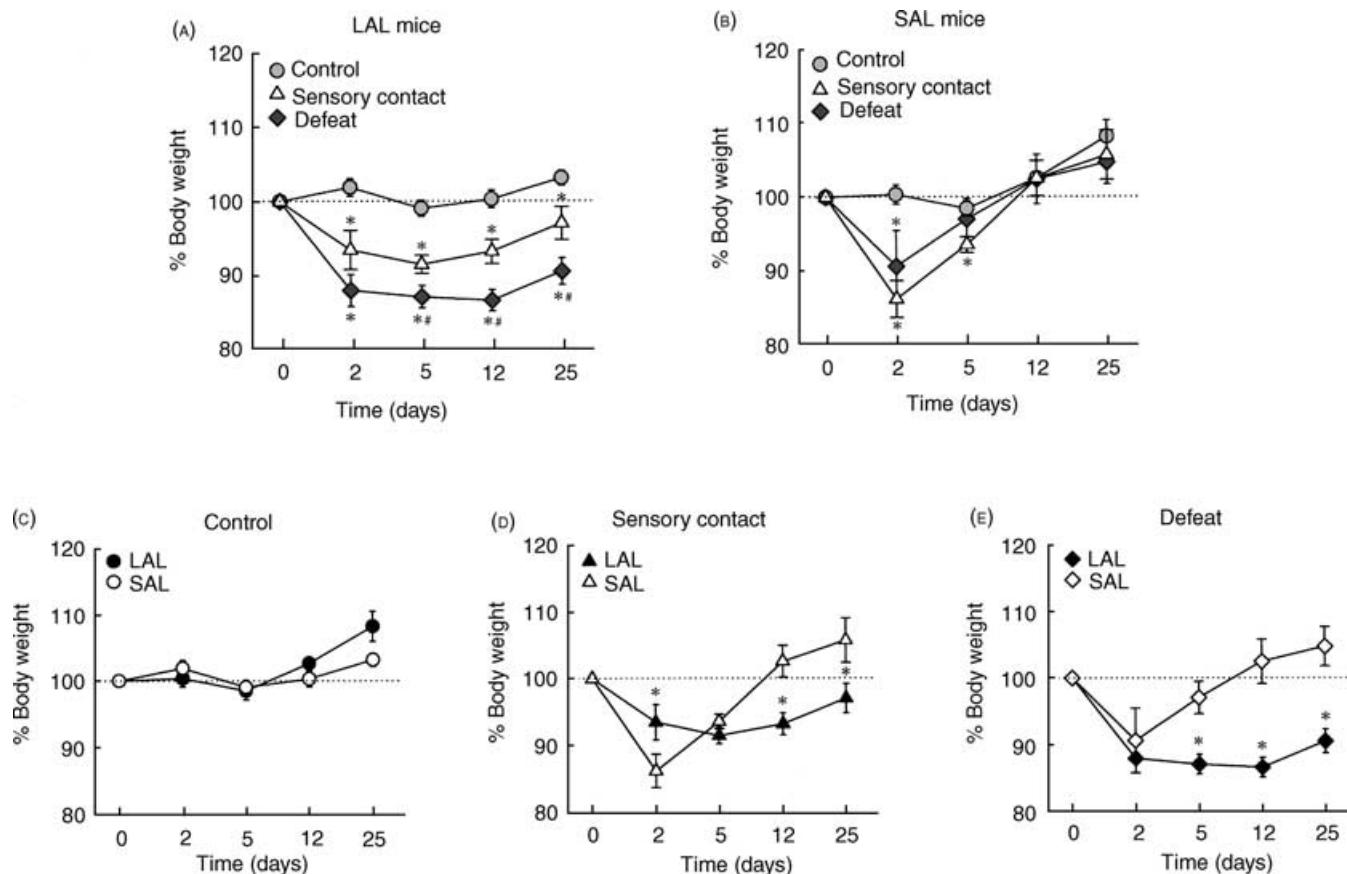


FIG. 2. Effect of sensory contact stress and defeat stress on body weight of nonaggressive (LAL) and aggressive (SAL) mice. At day 0, mice were housed in the partition cage. After 2 days of habituation, one group of mice was subjected to defeat for 21 consecutive days (defeat stress). Body weight is expressed as percentage of body weight at day 0. Treatment effects are found in LAL mice (A) and SAL mice (B). The same data is presented in a different form to show line effects in control mice (C), sensory contact mice (D) and defeated mice (E). *P at least <0.05 versus controls (A–B) or versus SAL mice (D–E), †P at least <0.05 versus sensory contact LAL mice (A), pairwise comparisons (LSD test) following repeated measures ANOVA.

Statistical analysis

Repeated measures analysis of variance (ANOVA) was used to determine line-, treatment- and interaction effects for body weight and behaviour. A paired sample *t*-test was used to compare behavioural trial 1 with trial 4 to determine a line-, treatment- and interaction effect over time. Plasma ACTH and corticosterone concentrations at day 25, relative organ weights, the mRNA expression of MR, GR and CRH and 5-HT_{1A} receptor binding were analysed by ANOVA to determine line-, treatment- and interaction effects. When a significance was revealed, appropriate pairwise comparisons (LSD test) were performed based on the estimated marginal means. For all tests, the statistical software package SPSS, version 9 (Chicago, IL, USA) was used. Data are presented as mean \pm SEM. $P < 0.05$ was considered statistically significant.

Results

Body weight (Fig. 2)

All experimental mice were weighed just before they were placed in the partition cage (day 0), and after 2, 5, 12 and 25 days in the partition cage. Control mice were weighed at the same days as the experimental mice. Body weight was expressed as percentage body weight measured at day 0. Overall effects were found for treatment ($F_{2,34} = 4.786$, $P < 0.05$), line ($F_{1,34} = 4.645$, $P < 0.05$) and treatment \times line ($F_{2,34} = 4.092$, $P < 0.05$).

Time \times treatment effect (Fig. 2A,B)

Housing mice in a partition cage for 25 days induced a significant time \times treatment effect ($F_{8,136} = 3.301$, $P < 0.005$) for body weight in both mouse lines.

Within the LAL line, a decrease in body weight was observed in both stress paradigms compared to controls (sensory contact: day 2, $P < 0.01$; day 5, $P < 0.001$; day 12, $P < 0.005$; day 25, $P = 0.050$; defeat: at all days, $P < 0.001$) (Fig. 2A). Furthermore, defeated LAL mice showed a significantly lower body weight than sensory contact LAL mice at day 5 ($P < 0.05$), day 12 ($P < 0.01$) and day 25 ($P < 0.05$) (Fig. 2A).

Within the SAL line, both stress paradigms induced a significant reduction in bodyweight compared to controls at day 2 (sensory contact: $P < 0.001$; defeat: $P < 0.01$) (Fig. 2B). At day 5, only sensory contact SAL mice showed a significantly lower body weight compared to controls ($P < 0.05$) (Fig. 2B).

Time \times line effect (Fig. 2C–E)

A significant time \times line effect ($F_{4,136} = 6.048$, $P < 0.001$) was found for body weight. Control SAL and LAL mice did not differ in body weight (Fig. 2C). Sensory contact stress induced in LAL mice a significantly lower reduction in body weight compared to SAL at day 2 ($P < 0.05$), but a significantly higher reduction in body weight at day 12 ($P < 0.001$) and day 25 ($P < 0.01$) (Fig. 2D). Repeated defeat induced a significantly lower body weight in LAL compared to SAL at days 5, 12 and 25 ($P < 0.001$) (Fig. 2E).

In summary, both mouse lines showed a significant decrease in body weight upon housing in the partition cage, but only LAL mice showed long-lasting body weight loss in both stress paradigms compared to SAL and control LAL mice.

Organ weights (Fig. 3)

After the experimental period of 25 days, all mice were decapitated and several organs were weighed as possible peripheral indicators of an altered HPA functioning.

Treatment effect

Treatment effects were observed for the relative weights of adrenals, thymus and spleen ($F_{2,32} = 27.049$, $P < 0.001$; $F_{2,32} = 11.171$, $P < 0.001$; $F_{2,32} = 15.228$, $P < 0.001$, respectively).

In LAL mice, repeated defeat induced an increase in relative adrenal weight compared to control LAL ($P < 0.001$) and sensory contact LAL ($P < 0.001$) (Fig. 3A). Relative thymus weight was significantly reduced in sensory contact LAL and in defeated LAL compared to controls ($P < 0.005$) (Fig. 3B). Relative spleen weight was significantly increased in defeated LAL compared to controls and sensory contact LAL ($F_{2,32} = 4.739$, $P < 0.01$) (Fig. 3C).

In SAL mice, repeated defeat induced an increase in relative adrenal weight ($P < 0.001$ versus control; $P < 0.001$ versus sensory contact) (Fig. 3A), a decrease in relative thymus weight ($P < 0.01$ versus control; $P < 0.01$ versus sensory contact) (Fig. 3B), and an increase in relative spleen weight ($P < 0.005$ versus control; $P < 0.001$ versus sensory contact) (Fig. 3C). Sensory contact stress induced a small but significant decrease in relative spleen weight compared to SAL controls ($P < 0.05$) (Fig. 3C).

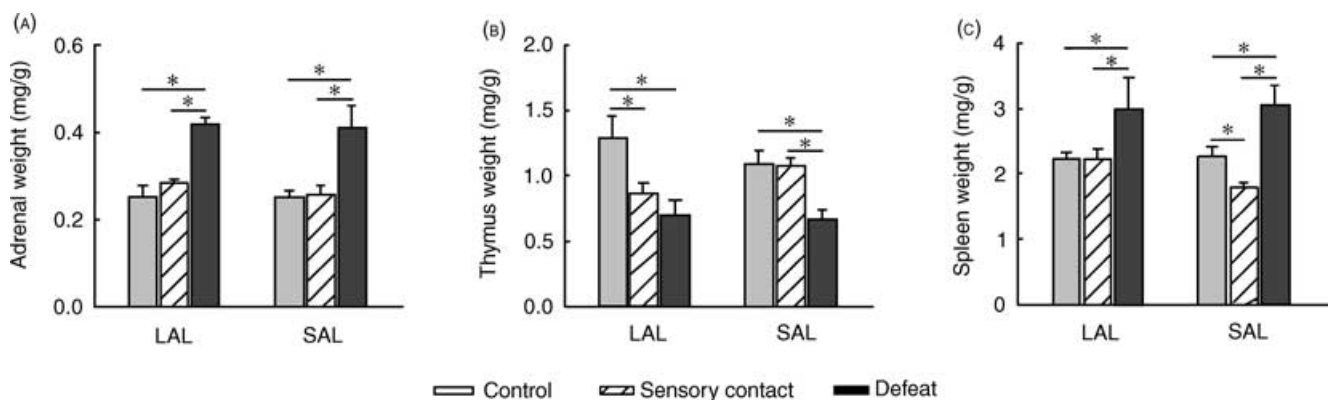


FIG. 3. Effect of sensory contact stress and defeat stress on the relative weights of adrenals, thymus and spleen of nonaggressive (LAL) and aggressive (SAL) mice. Treatment effects are shown for adrenal weight (A), thymus weight (B) and spleen weight (C) in LAL and SAL mice. No line differences were observed for relative organ weights. * P at least < 0.05 , pairwise comparisons (LSD test) following univariate ANOVA.

Line effect

No line effect was observed for the relative weight of adrenals, thymus and spleen.

In summary, the sensory contact stressor induced in LAL mice a decrease in thymus weight and in SAL mice a decrease in spleen weight. Repeated defeat induced in both mouse lines a significant increase in the relative weights of adrenals and spleen and a decrease in thymus weight.

Plasma corticosterone and ACTH (Fig. 4)

After the experimental period of 25 days, all mice were decapitated (4–3 h before lights off) and trunk blood was collected to measure plasma corticosterone and ACTH.

*Corticosterone (Fig. 4A,B)***Treatment effect**

A significant treatment effect ($F_{2,33} = 14.890$, $P < 0.001$) was found for plasma corticosterone concentrations. The sensory contact stressor induced a significant rise in corticosterone only

in LAL mice compared to LAL controls ($P < 0.01$) (Fig. 4A). Repeated defeat induced a significant increase in corticosterone concentration in both mouse lines (LAL mice: $P < 0.001$ versus control, $P < 0.01$ versus sensory contact LAL; SAL mice: $P < 0.05$ versus control and sensory contact SAL) (Fig. 4A).

Line effect

A significant line effect ($F_{1,33} = 5.225$, $P < 0.05$) was found for plasma corticosterone concentrations. The sensory contact stressor induced a higher concentration of corticosterone in LAL mice compared to SAL mice, but this difference just failed to reach significance ($P = 0.052$) (Fig. 4B). Repeated defeat induced a significantly higher increase in corticosterone in LAL mice than in SAL mice ($P < 0.05$) (Fig. 4B).

*ACTH (Fig. 4C,D)***Treatment \times line effect**

Plasma ACTH concentrations revealed a significant interaction effect ($F_{2,31} = 5.566$, $P < 0.01$). The sensory contact stressor induced in LAL mice a significant increase in ACTH concentration

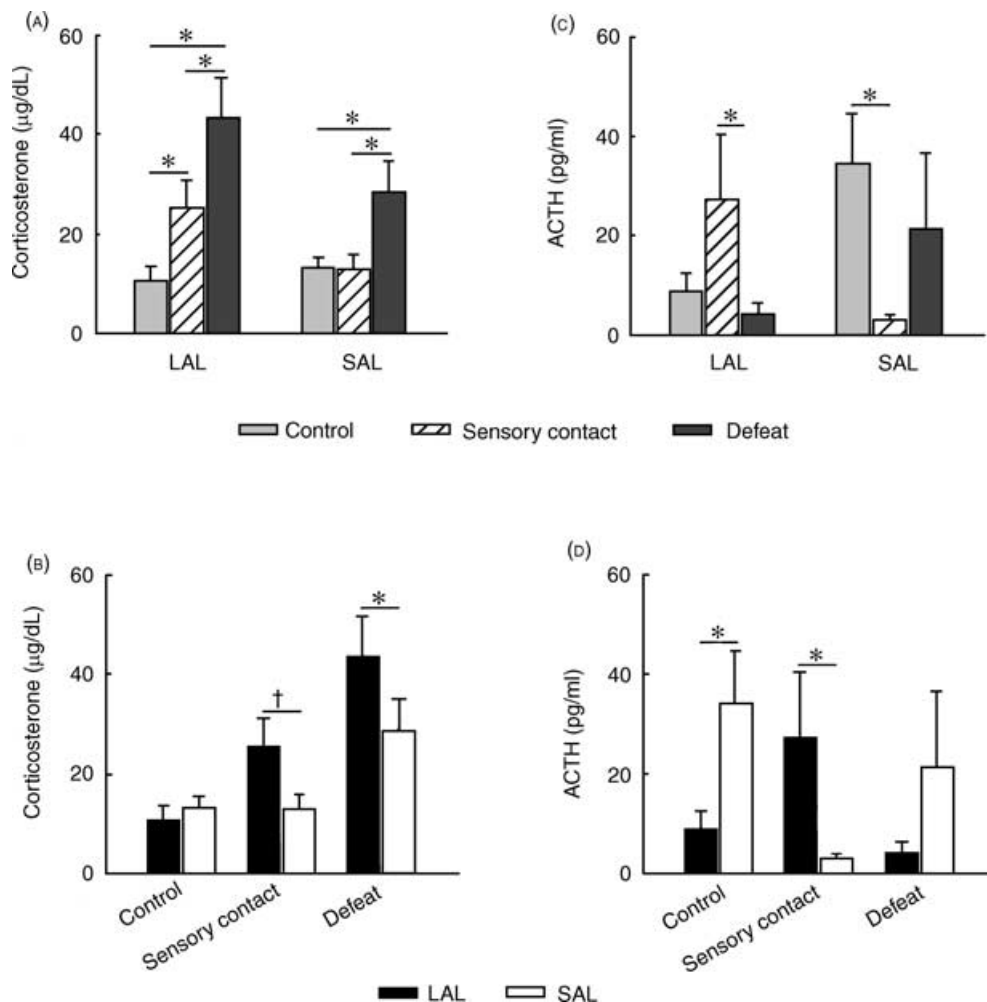


Fig. 4. Effect of sensory contact stress and defeat stress on plasma corticosterone and adrenocorticotrophic hormone (ACTH) concentrations measured at day 25 in nonaggressive (LAL) and aggressive (SAL) mice. Treatment effects were observed for corticosterone (A) and for ACTH (C) in both mouse lines. The same data are presented in a different form to show line effects within the treatment groups for corticosterone (B) and ACTH (D). * P at least < 0.05 , † $P = 0.052$, pairwise comparisons (LSD test) following univariate ANOVA.

compared to defeated LAL ($P < 0.05$) (Fig. 4c) whereas in SAL mice a significant decrease in ACTH concentration was found ($P < 0.05$ versus control) (Fig. 4c) ($P < 0.05$ versus LAL) (Fig. 4d). Control LAL mice showed significantly lower plasma ACTH concentrations than control SAL mice ($P < 0.05$) (Fig. 4d).

In summary, sensory contact stress induced in LAL mice a significant increase in plasma corticosterone concentrations compared to controls, and, in SAL mice, a significant decrease in plasma ACTH concentrations compared to controls and LAL mice. Repeated defeat induced a significant increase in plasma corticosterone in both mouse lines, although the concentrations were significantly higher in LAL mice.

MR, GR and CRH mRNA expression (Table 1)

The mRNA expression of MR, GR (in hippocampus) and CRH (in PVN) were measured after the experimental period of 25 days.

Hippocampal MR mRNA expression

A significant treatment effect was found for MR mRNA expression across all hippocampal subfields (dentate gyrus: $F_{2,27} = 6.993$, $P < 0.005$; CA1: $F_{2,27} = 8.706$, $P < 0.005$; CA2: $F_{2,27} = 8.013$, $P < 0.005$; CA3: $F_{2,27} = 5.397$, $P < 0.05$). The sensory contact stressor induced a reduction in MR mRNA expression in LAL mice compared to LAL controls and defeated LAL ($P < 0.001$ versus control and $P < 0.05$ versus defeated LAL for dentate gyrus and CA1 region (Table 1) ($P < 0.001$ versus control, $P < 0.05$ versus defeated LAL for CA2 and CA3 regions, data not shown). No line difference was found for hippocampal MR mRNA expression.

Hippocampal GR mRNA expression

GR mRNA expression in dentate gyrus and CA1 region of the hippocampus was not affected by treatment nor by line (Table 1).

Hippocampal MR:GR ratio

The MR:GR ratio in dentate gyrus and CA1 region of the hippocampus (determined by dividing the mRNA expression of MR by the mRNA expression of GR per mouse) showed a significant treatment effect ($F_{2,24} = 6.394$, $P < 0.01$ in dentate gyrus; $F_{2,24} = 6.250$, $P < 0.01$ in CA1). Both stress paradigms induced a significant decrease in MR/GR ratio in LAL mice

compared to controls (sensory contact: $P < 0.005$ in dentate gyrus and CA1; defeat: $P < 0.05$ in dentate gyrus and CA1) (Table 1).

Hypothalamic CRH mRNA expression

A treatment effect was observed for CRH mRNA expression in PVN ($F_{2,24} = 4.798$, $P < 0.05$). Defeated SAL mice showed a significantly higher CRH mRNA expression compared to control mice ($P < 0.05$) (Table 1). No line difference was observed for the mRNA expression of hypothalamic CRH.

In summary, sensory contact stress induced a significant decrease in hippocampal MR mRNA expression in LAL mice. Hippocampal GR mRNA expression did not show any significant treatment or line effect. Both stress paradigms induced a significant decrease in the hippocampal MR:GR ratio in LAL mice. Hypothalamic CRH mRNA expression was higher in defeated mice, reaching significance only in SAL mice.

5-HT_{1A} receptor binding (Table 2)

Binding to the 5-HT_{1A} receptor with [³H] 8-OH-DPAT was measured in dentate gyrus and CA1 subregions of the hippocampus, in the frontal cortex and the dorsal raphe nucleus of SAL and LAL mice. No significant treatment effect was observed in any brain region (Table 2). A line effect was observed in dentate gyrus ($F_{1,31} = 13.282$, $P < 0.005$) and CA1 region ($F_{1,30} = 37.277$, $P < 0.001$) of the hippocampus. LAL mice showed lower hippocampal 5-HT_{1A} receptor binding density than SAL mice, reaching a significantly difference in the dentate gyrus in sensory contact LAL compared to SAL ($P < 0.005$) (Table 2) and in the CA1 region in all LAL groups compared to SAL (sensory contact stress and defeat stress: $P < 0.001$; control: $P < 0.05$) (Table 2). No significant line difference in 5-HT_{1A} receptor binding density was observed in the frontal cortex nor in the dorsal raphe nucleus (Table 2).

Behaviour (Figs 5–8)

Behaviour was analysed at several time points to reveal possible time-dependent changes in behaviour induced by psychosocial stress. Four behaviour trials were performed for each behavioural test (trial 1 = 1 week before mice were placed in the partition cage; trial 2, 3 and 4 after 5, 12 and 23 days in the partition cage, respectively (i.e. after 3, 10 and 21 defeats, respectively).

TABLE 1. Effect of Sensory Contact Stress and Defeat Stress in Nonaggressive (LAL) and Aggressive (SAL) Mice on the mRNA Expression (In Arbitrary Units) of Mineralocorticoid Receptor (MR) and Glucocorticoid Receptor (GR) and MR:GR Ratio in the Dentate Gyrus (DG) and the CA1 Region of the Hippocampus, and the mRNA Expression of Corticotropin-Releasing Hormone (CRH) in the Paraventricular Nucleus (PVN) of the Hypothalamus.

Line	Treatment	MR		GR		MR:GR		CRH
		DG	CA1	DG	CA1	DG	CA1	PVN
LAL	Control	0.35 ± 0.01	0.30 ± 0.01	0.33 ± 0.03	0.32 ± 0.03	1.09 ± 0.10	0.93 ± 0.07	0.50 ± 0.03
LAL	Sensory contact	0.24 ± 0.02 ^{a,b}	0.19 ± 0.02 ^{a,b}	0.36 ± 0.01	0.34 ± 0.01	0.66 ± 0.07 ^d	0.55 ± 0.07 ^d	0.49 ± 0.04
LAL	Defeat	0.30 ± 0.02	0.25 ± 0.02	0.38 ± 0.01	0.37 ± 0.01	0.80 ± 0.06 ^c	0.69 ± 0.08 ^c	0.57 ± 0.04
SAL	Control	0.31 ± 0.02	0.27 ± 0.02	0.35 ± 0.01	0.35 ± 0.01	0.88 ± 0.05	0.77 ± 0.05	0.40 ± 0.03
SAL	Sensory contact	0.27 ± 0.04	0.23 ± 0.03	0.35 ± 0.03	0.35 ± 0.02	0.78 ± 0.08	0.64 ± 0.06	0.45 ± 0.09
SAL	Defeat	0.27 ± 0.04	0.22 ± 0.04	0.35 ± 0.04	0.34 ± 0.04	0.79 ± 0.18	0.66 ± 0.17	0.55 ± 0.03 ^e

^a $P < 0.001$ versus control, ^b $P < 0.05$ versus defeat, ^c $P < 0.05$ versus control, ^d $P < 0.005$ versus control, ^e $P < 0.05$ versus control, pairwise comparisons (LSD) following ANOVA.

TABLE 2. Effect of Sensory Contact Stress and Defeat Stress in Nonaggressive (LAL) and Aggressive (SAL) Mice on 8-OH-DPAT Binding (in fmol/mg tissue) to the 5-HT_{1A} Receptor in the Dentate Gyrus and CA1 Region of the Hippocampus, in the Frontal Cortex and in the Dorsal Raphe Nucleus (DRN)

Line	Treatment	Dentate Gyrus	CA1	Frontal Cortex	DRN
LAL	Control	11.5 ± 1.6	40.1 ± 2.6 ^c	8.4 ± 0.8	47.6 ± 1.4
LAL	Sensory contact	9.7 ± 0.6 ^a	35.9 ± 2.9 ^b	6.5 ± 0.7	40.9 ± 3.1
LAL	Defeat	11.9 ± 0.9	33.0 ± 2.8 ^b	5.3 ± 0.9	45.6 ± 0.8
SAL	Control	15.4 ± 4.0	50.0 ± 3.9	9.7 ± 1.8	39.3 ± 2.9
SAL	Sensory contact	22.1 ± 2.5	54.1 ± 3.6	6.9 ± 1.6	41.5 ± 3.8
SAL	Defeat	19.4 ± 5.4	52.1 ± 3.1	10.5 ± 3.9	41.7 ± 4.1

^aP < 0.005 versus SAL, ^bP < 0.001 versus SAL, ^cP < 0.05 versus SAL, pairwise comparisons (LSD) following univariate ANOVA.

Elevated plus-maze (Fig. 5)

Time × treatment effect

Neither stress paradigm induced a treatment effect.

Time × line effect

Line effects were observed for all behavioural parameters in the elevated plus-maze: percentage time spent on open arms ($F_{3,99} = 3.493$, $P < 0.05$); percentage of open arm entries ($F_{3,99} = 8.722$, $P < 0.001$); total entries ($F_{3,99} = 4.517$, $P < 0.01$). LAL mice spent less time on the open arms at trial 3 ($P < 0.05$) (Fig. 5A), showed a lower percentage of open arm entries ($P < 0.05$ at trials 2 and 4, $P < 0.01$ at trial 3) (Fig. 5B) and had a lower number of total entries ($P < 0.01$ at trials 1, 3 and 4) (Fig. 5C) than SAL mice.

Repeated behavioural testing

Repeated testing on the elevated plus-maze resulted in SAL mice in a significant increase in time spent on open arms and open arm entries ($P \leq 0.001$, trial 4 versus trial 1) (Fig. 5A,B), whereas LAL mice showed a significant decrease in the number of total entries ($P < 0.05$, trial 4 versus trial 1) (Fig. 5C).

Sudden silence test (Fig. 6)

Overall treatment effect

Behaviour in the sudden silence test of SAL and LAL mice revealed a small but significant overall treatment effect for grooming behaviour ($F_{2,32} = 4.194$, $P < 0.05$). The sensory con-

tact stressor induced in LAL mice a significant increase in grooming behaviour compared to control ($P < 0.05$ at trial 3, $P < 0.05$ at trial 4) and defeated mice ($P < 0.005$ at trial 3, $P < 0.05$ at trial 4, data not shown).

Overall line effect

Overall line effect were found for freezing ($F_{1,32} = 36.896$, $P < 0.001$), exploration ($F_{1,32} = 40.572$, $P < 0.001$), immobility ($F_{1,32} = 17.563$, $P < 0.001$) and digging ($F_{1,32} = 14.233$, $P < 0.005$). LAL mice showed significantly more freezing behaviour ($P < 0.01$ at trials 1, 3 and 4; $P < 0.05$ at trial 2) (Fig. 6A) and, as a consequence, less exploration behaviour ($P < 0.01$ at trials 1, 3 and 4) (Fig. 6B), and less digging behaviour ($P < 0.01$ at trials 1 and 3; $P < 0.05$ at trial 4) (Fig. 6E) than SAL mice.

Time × line effect

A time × line effect was found for grooming ($F_{3,96} = 3.072$, $P < 0.05$) and immobility ($F_{3,96} = 4.499$, $P < 0.01$). LAL mice showed significantly more grooming behaviour ($P = 0.050$ at trial 4) (Fig. 6C) and more immobility behaviour ($P < 0.05$ at trial 3 and $P < 0.01$ at trial 4) (Fig. 6D) than SAL mice.

Time × treatment × line effect

A small but significant time × treatment × line effect was found for digging behaviour ($F_{6,96} = 2.387$, $P < 0.05$). Both stress paradigms induced in SAL mice a decrease in digging behaviour compared to controls at trial 3 (sensory contact, $P < 0.005$; defeat, $P < 0.001$, data not shown).

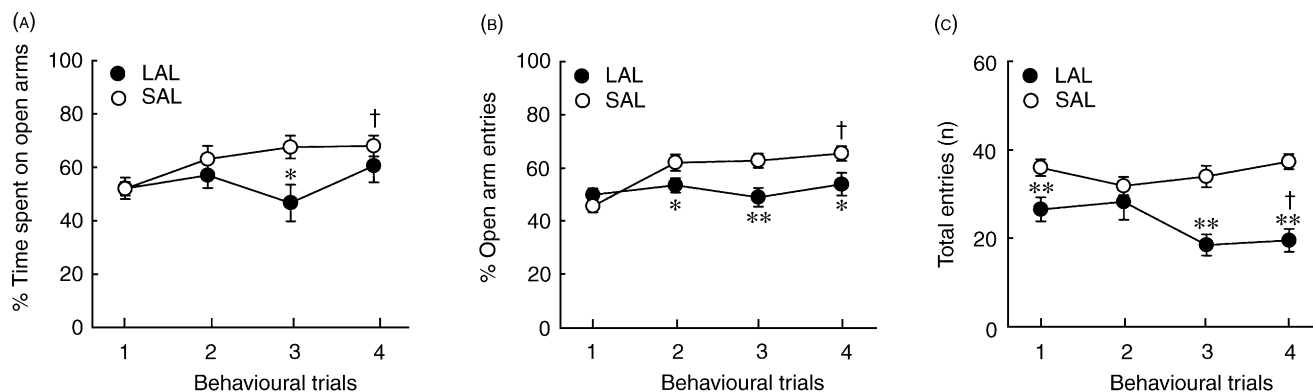


FIG. 5. Elevated plus-maze behaviour of nonaggressive (LAL) and aggressive (SAL) mice. Behavioural tests were performed one week before and after 5, 12 and 23 days in the partition cage (that is after 3, 10 and 21 defeats, respectively). Due to the lack of treatment effects, data for all LAL groups, as well as of all SAL groups, are merged to show line effects in percentage time spent on open arms (A), percentage of open arm entries (B) and total entries (C). * $P < 0.05$, ** $P < 0.01$ versus SAL mice, † P at least < 0.05 versus trial 1, pairwise comparison (LSD test) following repeated measures ANOVA.

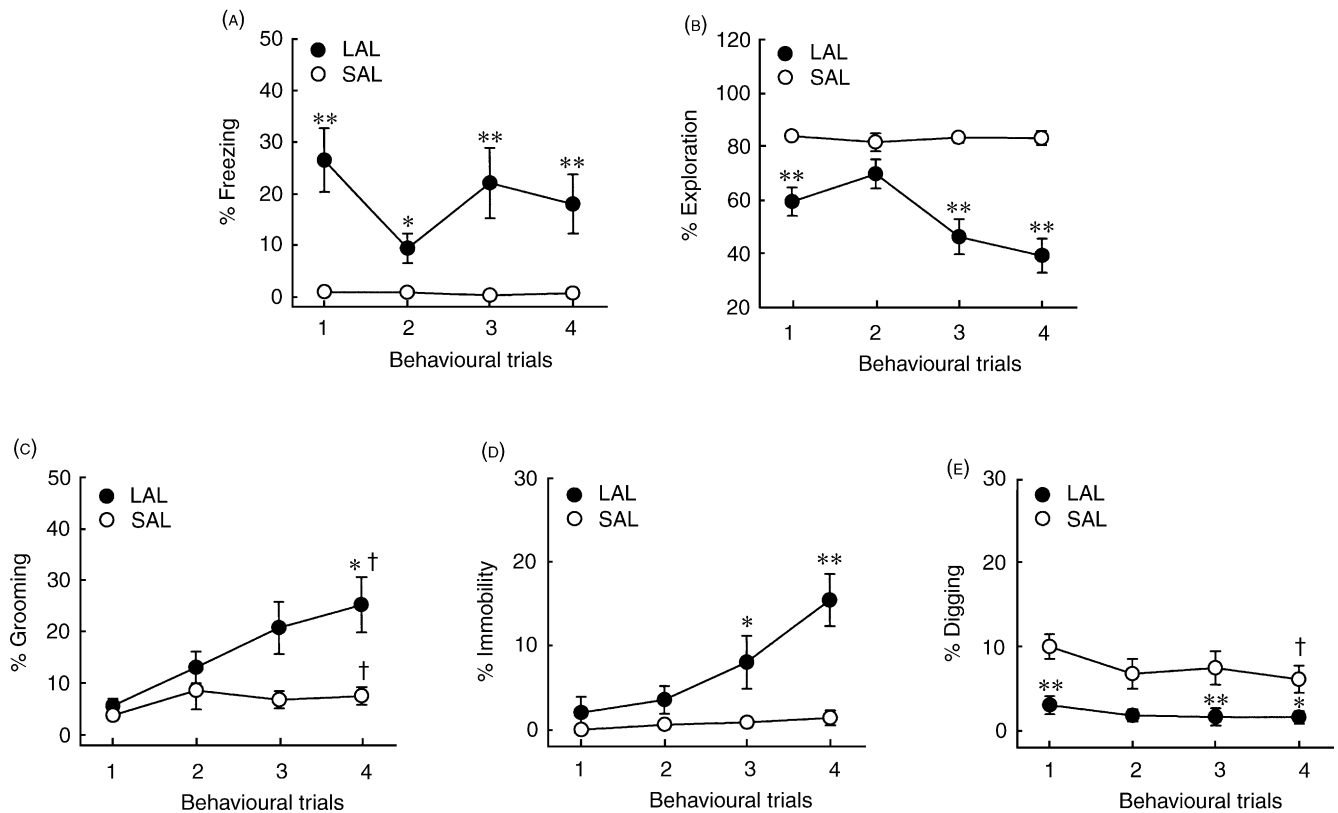


FIG. 6. Behaviour in the sudden silence test of nonaggressive (LAL) and aggressive (SAL) mice. Data for all LAL groups, as well as of all SAL groups, are merged to show line effects for freezing behaviour (A), exploration behaviour (B), grooming behaviour (C), immobility behaviour (D) and digging behaviour (E). * $P \leq 0.05$, ** $P < 0.01$ versus SAL mice, † P at least < 0.05 versus trial 1, pairwise comparison (LSD test) following repeated measures ANOVA.

Repeated behavioural testing

Repeated testing resulted in SAL mice in a significant increase in grooming behaviour ($P < 0.01$, trial 4 versus trial 1) (Fig. 6C) and a decrease in digging behaviour ($P < 0.05$, trial 4 versus trial 1) (Fig. 6E), whereas LAL mice showed a significant increase in grooming and immobility behaviour ($P < 0.005$, trial 4 versus trial 1) (Fig. 6C,D).

Open field (Fig. 7)

Overall treatment effect

An overall treatment effect was found for moving velocity ($F_{2,33} = 3.362$, $P < 0.05$). Moving velocity was significantly higher in sensory contact LAL ($P < 0.05$ at trial 1) and defeated LAL ($P < 0.005$ at trial 1, $P < 0.05$ at trial 2) compared to control LAL (data not shown).

Overall line effect

An overall line effect was found for immobility ($F_{1,33} = 30.659$, $P < 0.001$), moving velocity ($F_{1,33} = 14.278$, $P < 0.005$) and thigmotaxis ($F_{1,33} = 5.402$, $P < 0.05$). LAL mice showed significantly more immobility behaviour ($P < 0.005$ at trials 1, 3 and 4; $P < 0.05$ at trial 2) (Fig. 7B), higher moving velocity ($P < 0.005$ at trials 1, 2 and 4; $P < 0.05$ at trial 3) (Fig. 7C) and more thigmotaxis behaviour ($P < 0.05$ at trial 3) (Fig. 7D) than SAL mice.

Time \times treatment effect

A time \times treatment effect was found for total distance travelled ($F_{6,99} = 2.682$, $P < 0.05$), which was significantly higher in sen-

sory contact LAL ($P < 0.05$ at trial 1) and defeated LAL ($P < 0.005$ at trial 1, $P < 0.05$ at trial 2) compared to control LAL (data not shown). However, this behavioural difference was already present before treatment and was therefore an unfortunate baseline difference rather than a treatment difference.

Time \times line effect

A time \times line effect was found for total distance travelled ($F_{3,99} = 4.021$, $P < 0.05$) and immobility behaviour ($F_{3,99} = 3.526$, $P < 0.05$).

Time \times treatment \times line effect

A small but significant time \times treatment \times line effect was found for total distance travelled ($F_{6,99} = 2.209$, $P < 0.05$).

Repeated behavioural testing

Both mouse lines showed a significant decrease in total distance travelled ($P < 0.005$ and $P < 0.001$, respectively) (Fig. 7A), an increase in immobility behaviour ($P < 0.01$ and $P < 0.005$) (Fig. 7B) and a small decrease in moving velocity (both mouse lines $P < 0.05$) (Fig. 7C) by comparing behavioural trial 4 with trial 1.

Forced swim test (Fig. 8)

Overall treatment effect

An overall treatment effect was found for immobility behaviour ($F_{2,33} = 3.389$, $P < 0.05$).

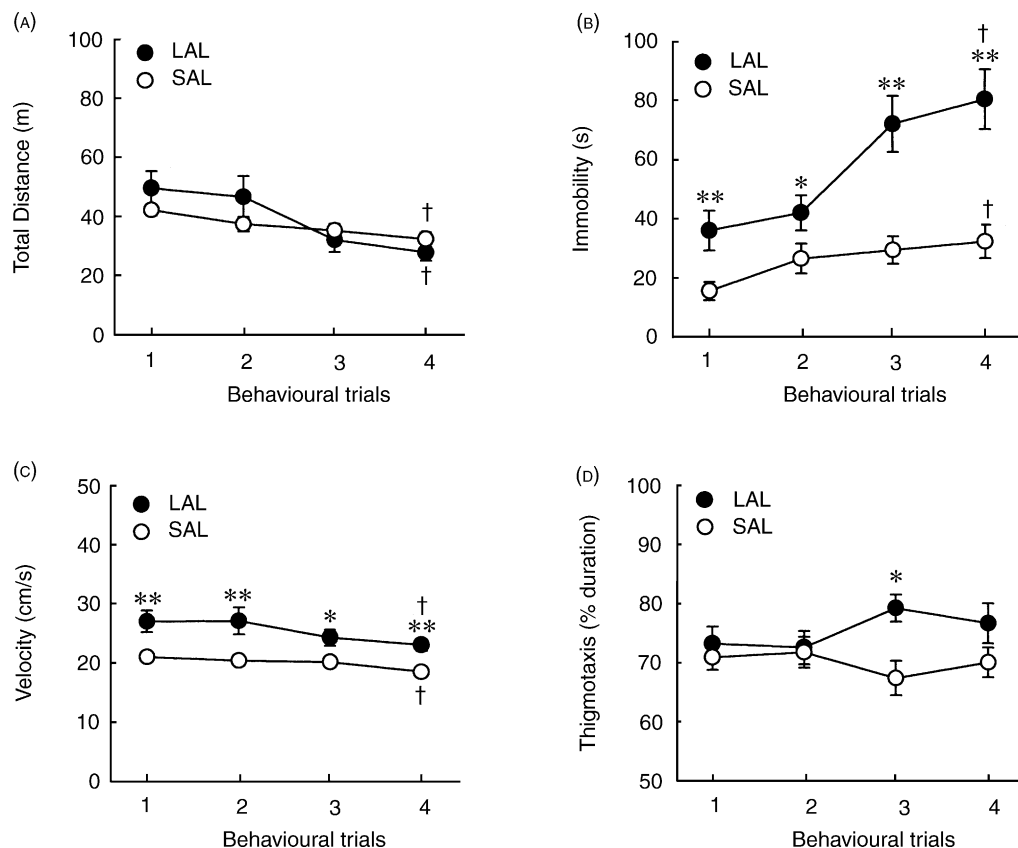


FIG. 7. Open field behaviour of nonaggressive (LAL) and aggressive (SAL) mice. Data for all LAL groups, as well as of all SAL groups, are merged to show line effects for total distance (A), immobility behaviour (B), moving velocity (C) and thigmotaxis (D). * $P < 0.05$, ** $P < 0.01$ versus SAL mice, † P at least < 0.05 versus trial 1, pairwise comparisons (LSD test) following repeated measures ANOVA.

Overall line effect

An overall line effect was found for immobility behaviour ($F_{1,33} = 13.911$, $P < 0.005$). LAL mice showed more immobility behaviour than SAL mice (trial 1: $P < 0.001$, trial 2: $P < 0.05$) (Fig. 8A).

Time \times treatment effect

A time \times treatment effect ($F_{3,99} = 3.226$, $P < 0.01$) was observed. Defeated LAL mice showed less immobility behaviour than

control LAL mice (trial 3: $P < 0.005$; trial 4: $P = 0.005$) (Fig. 8B) and sensory contact LAL mice (trial 4: $P < 0.05$) (Fig. 8B). Defeated SAL mice showed significantly more immobility behaviour than sensory contact SAL mice (trial 3: $P < 0.01$) (Fig. 8C).

Time \times treatment \times line effect

A time \times treatment \times line effect ($F_{2,33} = 4.688$, $P < 0.05$) was found for behaviour in the forced swim test.

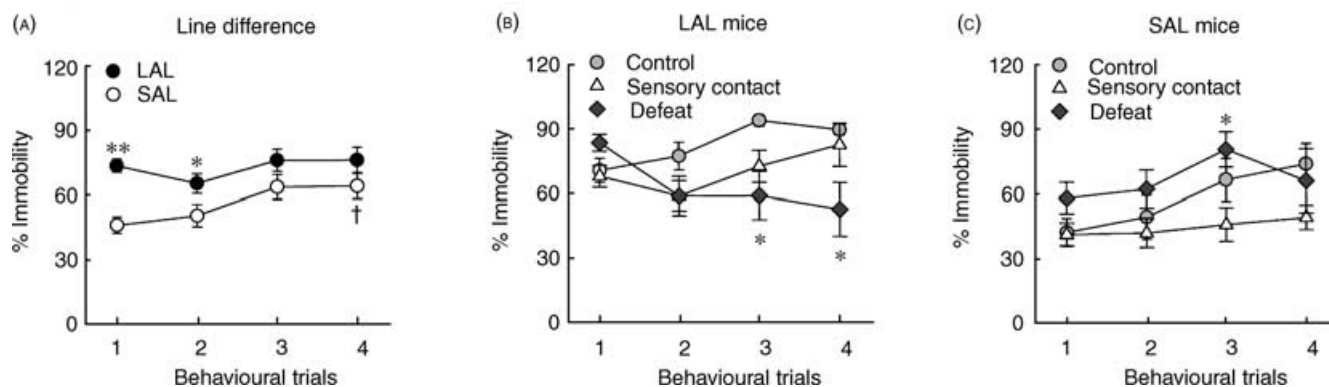


FIG. 8. Immobility behaviour during the forced swim test of nonaggressive (LAL) and aggressive (SAL) mice. Data for all LAL groups, as well as of all SAL groups, are merged to show line effects for immobility behaviour (A). A treatment effect was found for immobility behaviour in LAL mice (B) and in SAL mice (C). * P at least < 0.05 versus SAL mice, † $P < 0.005$ versus trial 1, pairwise comparisons (LSD test) following repeated measures ANOVA.

Repeated behavioural testing

Repeated forced swimming induced a significant increase in immobility behaviour in SAL mice only ($P < 0.005$, trial 4 versus trial 1) (Fig. 8A).

Summary of behaviour

Clear line differences were observed in all behavioural tests. LAL mice were less active than SAL mice on the elevated plus-maze, showed more freezing behaviour in the sudden silence test and were more immobile in the open field and forced swim test. Both stress paradigms induced minor, time-dependent and stressor-specific changes in behaviour. The sensory contact stressor caused in LAL mice an increase in grooming behaviour in the sudden silence test at trials 3 and 4 while, in SAL mice, a decrease in digging behaviour was observed but only at trial 3. The defeat stressor induced in LAL mice a decrease in immobility behaviour in the forced swimming test at trials 3 and 4 while, in SAL mice, an increase in immobility was observed only at trial 3 compared to sensory contact SAL mice. Repeated testing resulted in a decrease in activity of LAL mice on the elevated plus-maze and in the sudden silence test. In the open field, both mouse lines showed a decrease in activity. Repeated forced swimming induced an increase in immobility behaviour only in SAL mice.

Discussion

Our previous studies have demonstrated that male wild house-mice selected for a long attack latency (LAL) showed a higher stress-induced corticosterone output than short attack latency (SAL) mice (5). Therefore, we designed experiments to test the hypothesis that LAL mice are more susceptible to chronic stress than SAL mice. The present results demonstrate that SAL and LAL differ in their response to a chronic inescapable psychosocial stressor. Continuously living in sensory contact with a dominant male mouse caused long-lasting stress effects in LAL but not in SAL males, whereas repeated defeat induced an idiosyncratic pattern of stress symptoms in each of the two mouse lines.

The sensory contact stressor, with or without the defeat stressor, induced a prolonged decrease in body weight in LAL mice only. Social stress-induced decreases in body weight have been reported before (18, 19), and have been associated with hypophagia (20). Although food intake was not measured in our study, all mice had free access to food and hence differences in body weight may be due to line-specific differences in the anorexigenic effects of stress. In LAL mice, stress-induced body weight loss was associated with elevated levels of corticosterone and thymus involution. The defeat stressor in LAL mice induced significantly higher corticosterone secretion and adrenal hypertrophy compared to the sensory contact stressor, indicating that repeated defeat was a more severe stressor than adverse sensory contact. By contrast, both stress paradigms induced in SAL mice only a transient decrease in body weight, and the sensory contact stressor failed to induce an increase in corticosterone secretion. Although plasma concentrations of corticosterone were found to be elevated in defeated SAL mice compared to controls, the levels were significantly lower than in LAL mice. Collectively, the data show that in both stress paradigms, the reduction in body weight and the rise in corticosterone concentrations were much more profound in LAL mice than in SAL mice. LAL mice therefore showed a long-

lasting enhanced susceptibility to both stressors, with defeat being more severe than sensory contact.

The stress-induced elevated glucocorticoid concentrations in both mouse lines were not found to be associated with significant increases in plasma ACTH compared to control groups. Within the control groups, ACTH concentrations were significantly lower in LAL mice than in SAL mice, while corticosterone concentrations were similar, indicating a line difference in adrenocortical sensitivity to ACTH. This is consistent with previous findings (5). The sensory contact stressor induced higher ACTH secretion in LAL mice than in SAL mice and defeated LAL mice, but failed to induce a significant difference compared to control LAL mice. This may indicate that the corticosterone hypersecretion in sensory contact LAL mice was induced by increased adrenal sensitivity to ACTH [e.g. by increased splanchnic nerve stimulation (21)], or by ACTH-independent mechanisms stimulating corticosterone secretion [e.g. by vasoactive intestinal peptide released from the adrenal medulla following splanchnic nerve stimulation (22)]. The discrepancy between ACTH and corticosterone in both defeated SAL and LAL mice may be explained by adrenal hypertrophy. Although no alterations in ACTH concentrations were found, hypothalamic CRH mRNA expression was significantly higher in defeated SAL mice than in controls. If this increase is associated with increased CRH bioavailability, the absence of elevated ACTH secretion in defeated SAL mice may suggest adaptations at the pituitary level. Together, these data show that both stress paradigms induced line-specific and stressor-specific adaptations at the pituitary and/or adrenal level.

Brain corticosteroid receptors play a critical role in the behavioural reactivity and adaptation to stress (23). In particular, a balance in MR- and GR-mediated events in the hippocampus is thought to be necessary to maintain homeostasis and to protect the brain against stress-related brain disorders (24, 25). Therefore, the effect of the two stress paradigms on hippocampal MR and GR mRNA expression was determined in SAL and LAL mice. Only in LAL mice, sensory contact stress induced a significant decrease in MR mRNA, whereas repeated defeat induced more subtle changes in MR and GR mRNA expression, both resulting in a significant lower hippocampal MR : GR ratio compared to controls. Similar stress-induced long-term decreases in MR mRNA expression and MR/GR ratio have been reported previously (24, 26–28). Furthermore, it was found that suicide victims with a history of depression showed a significant lower hippocampal MR : GR ratio (26). A reduced capacity of especially hippocampal MRs has been hypothesized to be involved in the HPA system dysregulation found in human depression (29, 30). Accordingly, a number of studies, including the present one, showed that some neuroendocrine features found in human depression could be mimicked in rodent models by exposing mice to chronic psychosocial stress. We demonstrated that stress-induced hyperactivity in corticosterone output was associated with a change in MR : GR ratio in LAL mice but not in SAL mice. These data indicate that a genetic trait in coping style determines stress-induced alterations in hippocampal MR : GR ratio and further support that particularly LAL mice are susceptible to a psychosocial stressor.

The present study confirmed our previous finding (6) that LAL mice have a lower hippocampal 5-HT_{1A} receptor binding compared to SAL. It is known that hippocampal 5-HT_{1A} receptors can be modulated by corticosteroids and by exposure to stress (16, 26, 31). In this study, chronically elevated levels of corticosterone

induced by sensory contact stress in LAL mice and by defeat stress in both mouse lines did not induce a change in 5-HT_{1A} receptor binding in the hippocampus, nor in the frontal cortex or dorsal raphe nucleus. This is in contrast with the findings of Lopez *et al.* (26). Four weeks of chronic unpredictable stress induced a down-regulation in hippocampal 5-HT_{1A} receptors (26). However, the stress effect was rather small, especially when compared to the line difference in this study. Moreover, the severity and type of stressor may be an important factor in mediating changes in 5-HT_{1A} receptor regulation as, for example, daily swim stress for up to 3 weeks did not affect 5-HT_{1A} receptor binding (26). Thus, this study failed to demonstrate that, in SAL and LAL mice, hippocampal 5-HT_{1A} receptor protein can be down-regulated by the psychosocial stressors.

Line differences in behaviour were found in all four behavioural tests. In general, LAL mice were less active than SAL mice on the elevated plus-maze, showed more freezing behaviour in the sudden silence test, and were more immobile in the open field and forced swimming test, indicating a passive coping style displayed by LAL mice. Repeated behavioural testing resulted in a further decrease in activity of LAL mice in the elevated plus-maze and sudden silence test. In the open field, both mouse lines showed decreases in activity, suggesting locomotor habituation. Repeated forced swimming induced an increase in immobility in SAL mice, thereby eliminating the initial line difference in immobility behaviour. Both stress paradigms induced mild, stressor-specific changes in the behaviour of LAL mice, whereas in SAL, defeat-induced stress symptoms were not associated with a consistent behavioural change. In LAL mice, 12 days of sensory contact stress induced a consistent increase in grooming behaviour in the sudden silence test compared to the other LAL groups. Self-grooming is associated with HPA axis activity and is believed to reduce arousal after exposure to a stressor (32). After 10 and 21 defeats, LAL mice showed a decrease in immobility behaviour in the forced swim test compared to SAL and control LAL. High immobility behaviour in this test has been associated with a state of behavioural despair, and antidepressants were shown to successfully reduce this behaviour (15). Therefore, a stress-induced increase rather than a decrease in immobility behaviour was expected in this study. However, LAL mice had significantly higher immobility scores than SAL mice before treatment, indicating that the experience of repeated defeat induced a change in the behavioural strategy of LAL mice. Flexibility in behavioural strategy have been reported before in two mouse strains in which a short period of food shortage reversed strain differences in behavioural responses to amphetamine (33). Nevertheless, the stress-induced behavioural changes in this study were rather small, especially when compared to the line differences in behaviour. This may indicate that mice originating from genetic selection for many generations have lost a certain flexibility in their behavioural responses. In addition, repeated testing using the same behavioural paradigms can have an impact on, for example, anxiety measurements (34). As a result, behavioural experience due to repeated testing may have interfered with stress-induced behavioural alterations. Together, our data indicate that SAL and LAL mice showed clear differences in behaviour, which were generally still present after repeated testing, and that both stress paradigms induced small but consistent behavioural changes only in LAL mice.

In conclusion, the present data show that genetic selection for coping style predicts stressor susceptibility. The two stress para-

digms, sensory contact stress and defeat stress, induced distinctly different physiological and neuroendocrine alterations in LAL compared to SAL mice. Particularly in the sensory contact paradigm, LAL mice showed stress-sensitivity, as characterized by a long-lasting decrease in body weight, persistently elevated plasma ACTH and corticosterone levels and a lower hippocampal MR:GR ratio. LAL mice subjected to this stress paradigm therefore present an intriguing opportunity to link genetic factors with coping styles and stressor susceptibility. Indeed, using a genomic approach, we recently identified in LAL mice subjected to the sensory contact stressor a gene pattern encoding key elements in signalling cascades underlying stress-induced changes in synaptic plasticity (D. Feldker, personal communication).

Acknowledgements

The authors thank Gerardus Zuidema and Auke Meinema for excellent mouse care, Jan Bruggink for technical assistance and Mathias Schmidt for kindly providing labelled CRH probe. A.H. Veenema was supported by the Netherlands Organization for Scientific Research grant NWO-MW 940-70-005.

Accepted 18 November 2002

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